Research Article

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Role of Hep Par1 Immuno marker for Cytological Differentiation between Primary and Metastatic Liver Cancer

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ABSTRACT

Space occupying lesions of liver is a major health problem worldwide. Among them the most important is malignant SOLs. Discrimination between primary HCC and metastatic tumor is extremely important for treating the patient. For this purpose immunocytochemical technique can be applied on fine needle aspiration samples. Among the various immunological markers, the role of Hepatocyte Paraffin 1 antibody (Hep Par 1) is found to be very much convincing for its higher sensitivity and specificity.

To categorize the malignant lesions into primary and metastatic by immunocytochemistry, this cytomorphological study was done on patients of radiologically diagnosed space occupying lesions of liver. The study population comprised of benign and malignant hepatic SOL cases attending at Department of Pathology and Hepatology, DMCH over a period of two years (July 2017 to June 2019). The total number of samples was 100. Fine needle aspiration was done and cell block was prepared from residual material. Immunocytochemistry with Hep Par1 was applied on cell block material of malignant lesions. Cytomorphological and immunocytochemical findings of each cases were documented in details. The data were collected and statistical analysis was done by SPSS.

The mean age of study patients were found 47.0±13.37 years in benign and 53.63±10.89 years in malignant group with age ranging from 26 to 78 years. Male to female ratio was 4:1. According to cytomorphology, 32(50.8%) cases were diagnosed as hepatocellular carcinoma, 25(39.7%) as metastatic adenocarcinoma, 1(1.6%) metastatic small cell carcinoma and 1(1.6%) metastatic spindle cell sarcoma. According to immunocytochemistry, diffuse cytoplasmic granular staining with Hep Par1 were found in 26(81.3%) and no staining was seen in 24(88.9%) cases. Sensitivity, specificity, PPV, NPV and accuracy of HepPar1 for detection of hepatocellular carcinoma were100%, 80%, 81.25%, 100% and 89.29% respectively. HepPar1 in conjunction to cytology is a very useful diagnostic modality in differentiating HCC from metastatic tumor in suspicious cases.

Keywords
Liver SOL, Immunocytochemistry, Hep Par1.

Introduction
Evaluation and management of hepatic space occupying lesions is a common clinical problem and their appropriate clinical management depends on accurate diagnosis. There are several diagnostic procedures to obtain preoperative tissue diagnosis to guide subsequent therapy. They include image guided fine needle aspiration cytology, blind percutaneous needle core biopsy and trans jugular needle core biopsy [1]. In contrast to core biopsy, fine needle aspiration cytology (FNAC) is a rapid, inexpensive and minimally invasive technique for diagnosis of liver space occupying lesions without significant complications. The main difficulties with cytologic diagnosis of liver SOLs are differentiating HCC from other carcinomas. These problems may be overcome by the application of immunocytochemical panels in cell block preparations from FNA sample. Various immunological markers have been used for the identification of these tumors that include α-1-antitrypsin, polyclonal Carcinoembryonic Antigen (p-CEA), cytokeratin 18, 7, 20, anti-AFP, CA19-9, CD10 etc. [2]. Wennerberg et al., [3] initiated the development of a monoclonal antibody known as Hepatocyte Paraffin 1 (Hep Par 1), which was produced in mice using tissue from a failed allograft liver. A single clone was isolated, which is specific for adult and fetal liver tissues. Hep Par 1 reacts with normal and neoplastic hepatocytes in routine formalin-fixed, paraffin-embedded material, producing a distinct granular, cytoplasmic staining of hepatocytes [4]. The advantages of this marker is its high sensitivity and specificity (both> 80%) [5]. The aim of this study wasto differentiate the primary and malignant lesions using Hep par 1 in cell block preparations from FNA samples. Thus to prevent the morbidity and mortality from primary and metastatic liver malignancy.

Materials and Methods
This cross sectional study was carried out in Department of Pathology, Dhaka Medical College from July 2017 to June 2019 to evaluate the role of Hep Par 1 immunomarker for differentiating primary and metastatic hepatic lesions in cell block samples. For this purpose, a total of 100 patients radiologically diagnosed as hepatic SOL and physically fit to sustain the FNAC procedure in the above mentioned hospital were included in this study. Under guidance of ultrasonography, FNAC was performed. After smear preparation from the fine needle aspirates on the glass slides, the residual material was routinely processed as cell block. The patients were divided into benign and malignant group according to cytomorphological pattern. Subsequently, immunocytochemistry was performed on cell block sections of malignant lesion using HepPar1 immuno marker. The final diagnosis was made on the basis of cytomorphology of neoplastic cells, clinico-radiological correlation, serum tumor markers, cell block asessment and immunocytochemistry with Hep par1 immuno marker on cell block sections. For diagnostic interpretation of immunocytochemical staining, a subjective, semiquantitative evaluation scheme was used based on the frequency of stained tumor cells described by Onofre et al. [6].

Results
Table 1 shows the distribution of the study patients according to age in malignant group. It was observed that 12 (37.5%) patients belonged to age 51-60 years in HCC and 16(59.3%) in MT. 50% of dysplasia patients are belong to 41-50 years age group. The mean age was found 52.75±11.47 years in HCC, 54.85±10.26 years in MT and 52.50±12.58 years in Dysplasia.

Table 2 shows the distribution of the study patients according to immunocytochemistry. Diagnosis in malignant group. It was observed that 26(81.3%) patients diagnosed as HCC and 24(88.9%) patients as metastatic tumor. 6(18.8%) patients diagnosed as HCC in cytology, showed negative staining in ICC. 3(11.1%) cases had inadequate material for ICC. According to semiquantitative evaluation scheme based on the frequency of stained tumor cells, positive staining with Hep Par1 were observed in 21%- 40% cells of 5 HCC cases, 41%- 60% cells of 7 HCC cases, 61%- 80% cells of 8 HCC cases and 81%- 100% of 6 HCC cases (Table 3).

Table 4 shows that, among 32 cytologically diagnosed hepatocellular carcinoma, ICC showed true positive in 26 cases and false positive in 6 cases, true negative in 24 cases. There were no false negative case according to ICC findings.

Table 5 shows that, cytologically diagnosed metastatic carcinoma were true positive in 24 cases out of 56, true negative in 26 cases, false negative in 6 cases. There were no false positive cases according to ICC.

Table 6 shows the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of Hep Par1 for diagnosis of HCC and MT.

Table 1: Distribution of the study patients according to age (n=63).

Age (years)	HCC (n=32)		MT (n=27)		Dysplasia (n=4)		P value
	n	%	n	%	n	%	
≤30	2	6.3	1	3.7	0	0.0	
31-40	3	9.4	2	7.4	1	25.0	
41-50	7	21.9	5	18.5	2	50.0	
51-60	12	37.5	16	59.3	0	0.0	
61-70	7	21.9	2	7.4	1	25.0	
71-80	1	3.1	1	3.7	0	0.0	
Mean ± SD	52.75	±11.47	54.85	±10.26	52.50	±12.58	0.750^{ns}

ns=not significant

Table 2: Distribution of the study patients according to immunocytochemistry (by HepPar1 (n=63).

ICC diagnosis	HCC (n=32)		MT (n=27)		Dysplasia (n=4)		P value
	n	%	n	%	n	%	
Hepatocelluar carcinoma	26	81.3	0	0.0	0	0.0	0.001^{s}
Metastatic carcinoma	0	0.0	24	88.9	0	0.0	
Negative for HCC	6	18.8	0	0.0	0	0.0	
Inadequate cell block	0	0.0	3	11.1	4	100.0	

s=significant
p value reached from Chi-square test
ICC= immunocytochemistry

Table 3: Distribution of study patients according to staining pattern of Hep Par1 immunomarker in HCC (n=26).

Staining of cells (%)	Score	No.	Percentage(%)
21-40	2	5	19.2
41-60	3	7	26.9
61-80	4	8	30.8
81-100	5	6	23.1

Table 4: Comparison between cytological and immunocytochemical diagnosis of hepatocellular carcinoma (n=56).

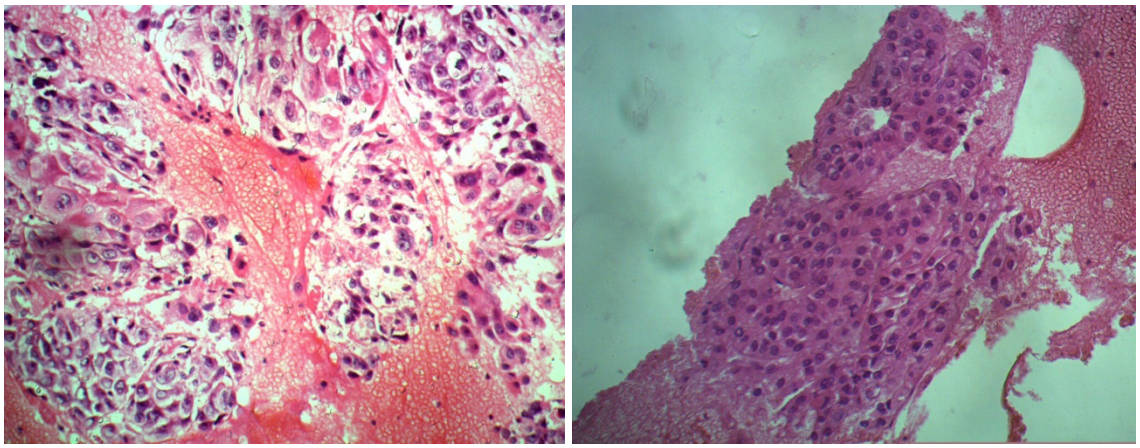
Diagnosis in cytology	Diagnosis in ICC	
	HCC positive (n=26)	HCC negative (n=30)
Positive for HCC (n=32)	26 (True positive)	6 (False positive)
Negative for HCC (n=24)	0 (False negative)	24 (True negative)

Table 5: Comparison between cytological and immunocytochemical diagnosis of metastatic tumor (n=56).

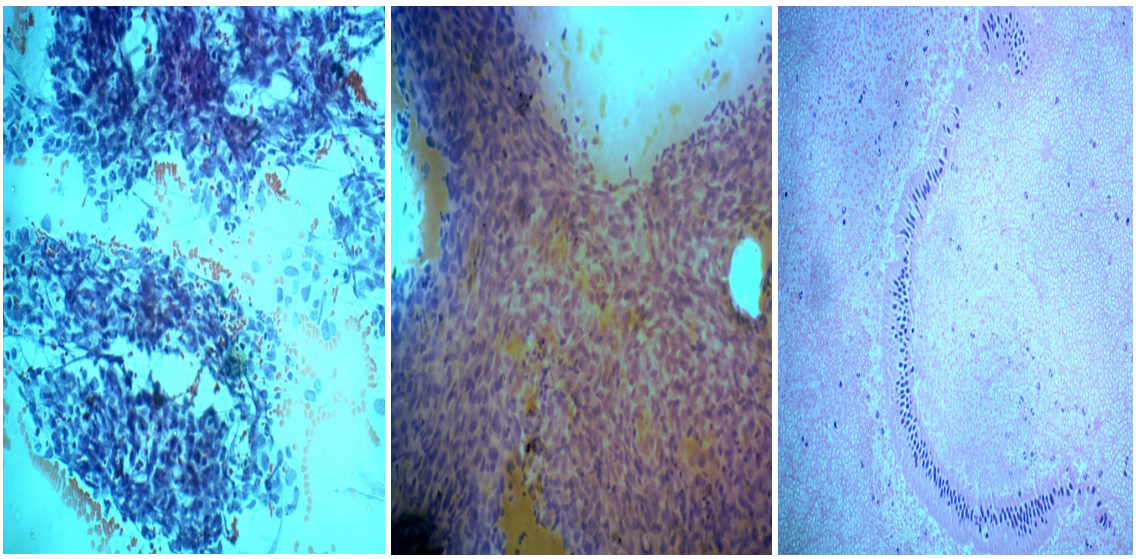
Diagnosis in cytology	Diagnosis in ICC	
	HCC positive (n=30)	HCC negative (n=26)
Positive for MT (n=24)	24 (True positive)	0 (False positive)
Negative for MT (n=32)	6 (False negative)	26 (True negative)

Table 6: Sensitivity, specificity, accuracy, positive predictive value and negative predictive value of Hep Par1 for diagnosis of HCC and MT.

Test of validity	HCC	MT
Sensitivity	100.00	80.00
Specificity	80.00	100.00
Accuracy	89.29	89.29
Positive Predictive Value	81.25	100.00
Negative Predictive Value	100.00	81.25



Figures 1 and 2: Photomicrograph of cell block of HCC. (Case no. 16 & case no 28 respectively, Hematoxylin and eosin stain, x200).



Figures 3, 4 and 5: Photomicrograph of cytosmear showing metastatic small cell carcinoma, metastatic spindle cell sarcoma, metastatic adecarcinoma, showing glandular pattern respectively (Case no. 14, x200, Case no. 38, x200, Case no. 11, x200).

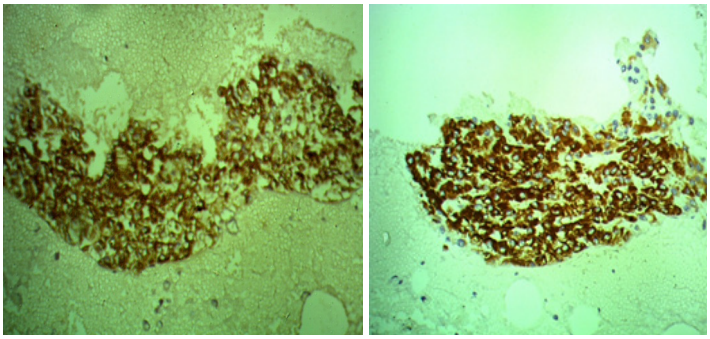


Figure 6 and 7: Photomicrograph of HCC showing positive staining for Hep Par 1 (Case no. 39& Case no. 87 x200 respectively).

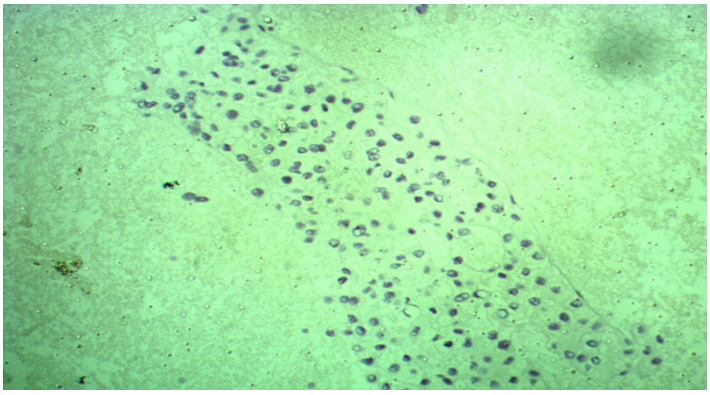


Figure 8: Photomicrograph of immunostaining of metastatic adenocarcinoma, showing negative staining with Hep par 1 (Case no. 60, x200).

Discussion

In this study Hep par1 antibody was applied on cell blocks prepared from malignant aspirates. Diffuse cytoplasmic granular staining were found in 26(81.3%) therefore they were diagnosed as hepatocellular carcinoma. No staining was seen in 24(88.9%) cases, therefore they were diagnosed as metastatic tumors. Negative staining also found in 6(18.8%) cases which were cytologically diagnosed as hepatocellular carcinoma. Onofre et al. found positive staining with HepPar1 in 100% HCC cases and negative staining in 100% metastatic carcinoma in their study. They concluded that HepPar1 is an excellent immunocytochemical marker for HCCs on smeared cells. Similarly, Lugli et al. [7] found positivity in 35 of 48 HCCs (73%), Chu et al. [8] in 88 of 96 HCCs (92%), and Lee et al. [9] in 60 of 75 HCCs (80%). Siddiqui et al. found high specificity and slightly lower sensitivity of Hep par1 for the identification of the hepatocellular phenotype. They also identified HepPar1 as an excellent immunocytochemical marker on the FNA cell block material.

In the present study, the staining pattern of Hep par1 in 26 HCC cases were analyzed. For diagnostic interpretation of immunocytochemical staining, a subjective, semiquantitative evaluation scheme was used based on the frequency of stained tumor cells described by Onofre et al. [6]. According to this scheme, scoring system was applied on all positive cases. Hepatocellular carcinoma was confirmed when 20% or more cells show diffuse, cytoplasmic granular staining [6]. In this study, out of 26 HCC cases 5(19.2%) cases showed score 2(21%-40% positively stained cells), 7(26.9%) cases showed score 3(41%-60%) positively stained cells, 8(30.8%) cases showed score 4(61%-80% positively stained cells) and 6(23.1%) cases showed score 5(81%-100% positively stained cells). Onofre et al. [6] observed score 4 in 83% cases and score 2 in100% cases in their study. However Siddiqui et al. [10] evaluated 50 HCC cases out of 75 cases, according to moderate to strong positive HepPar1 staining profile. Shiran et al. observed 23 out of 28 HCC with moderate to strong intensity in more than 10% of tumor cells. They calculated sensitivity of Hep par1 to diagnose HCC according to this level.

This study showed that, among 32 cytologically diagnosed hepatocellular carcinoma, ICC showed true positive in 26 cases and false positive in 6 cases, true negative in 24 cases. There were no false negative case according to ICC findings. Again cytologically diagnosed metastatic carcinoma were true positive in 24 cases out of 56, true negative in 26 cases, false negative in 6 cases. There were no false positive cases according to ICC.

In the present study, sensitivity, specificity, PPV, NPV and accuracy of HepPar1 for detection of primary and metastatic tumor of liver were analyzed. It was observed that, Hep par 1 was highly sensitive (100%) marker for detection of hepatocellular carcinoma. Specificity is slightly lower (80%) for detection of hepatocellular carcinoma. PPV and NPV of HepPar1 for detection of HCC is 81.25% and 100% respectively. In the contrary, Hep Par 1 was found to be highly specific marker for detection of metastatic tumor of liver. However sensitivity was found to be slightly lower (80%). PPV and NPV of HepPar1 for detection of metastatic tumor was 100% and 81.25% respectively. Overall accuracy of HepPar1 for detection of hepatocellular carcinoma and metastatic tumor was found to be 89.29 %. Nearly similar result was observed by Onofre et al. [6] who found 100% accuracy of Hep Par1 to differentiate HCC from metastatic carcinoma. A study to examine the sensitivity and specificity of Hep Par 1 antibody as a marker to distinguish HCC from metastatic carcinoma by Shiran et al. [4] found 82.1% sensitivity and 93.3% specificity of Hep Par 1 in distinguishing HCC from metastatic carcinoma, with a positive predictive value of 92.0 %. Similarly, in the original paper describing the antibody, Wennerberg et al. [3] found 37 of 38 HCCs to be positive and Leong et al. found 30 out of 32 HCCs to be positive. Kaker et al. [5] found >80% sensitivity and specificity of Hep par 1 to differentiate HCC from metastatic carcinoma. Siddiqui et al. found positive HepPar1 antibody in 50 of 50 HCC cases (100%). The positivity was cytoplasmic, diffuse, and granular.

Conclusion

This study was undertaken to evaluate the role of Hepatocyte Paraffin 1 antibody (Hep Par I) in differentiating primary and metastatic carcinoma. Discrimination between primary HCC and metastatic carcinoma is extremely important for treating the

patient. For this purpose immunocytochemical technique can be applied on fine needle aspiration samples. This study have found the convincing role of Hep Par 1 as an immunomarker for differentiating primary and malignant lesions of liver. So, it can be concluded that, immunocytochemistry using HepPar1is a very useful diagnostic modality in differentiating HCC from metastatic carcinoma in suspicious cases.

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